soaked in pH 8.8 buffer containing, per liter, 23.8 mmol of Tris, 6.69 mmol of barbituric acid, and 23.6 mmol of sodium barbiturate buffer. A 6-μL volume of serum was applied and the electrophoretic run was performed according to the conditions used for serum proteins (7), with the same buffer in the electrode tanks, for 20 min at 20 °C under a potential of 200 V.

After completion of the run, every strip was sandwiched between two Whatman no. 1 filter-paper strips soaked in the reagent mixture prepared from the commercial "optimized kinetic UV-test CK-NAC" (J. T. Baker Chem Co., Deventer, The Netherlands) according to the specifications of the manufacturer. The "sandwiches" were incubated for 12 min at 37 °C in an incubator saturated with water. The fluorescence of the isoenzyme bands on the cellulose acetate strips was measured in a Farrand MK 1 spectrofluorometer equipped with a thin-layer plate attachment and an Ankersmit A440 recorder (excitation 355 nm, emission 480 nm). The percentages of isoenzymes were calculated from the areas of the peaks, and the activity of each isoenzyme was deduced from the total activity measured with the CK-NAC Baker test at 30 °C.

To test the repeatability of the technique, the same technician processed several sera 12 times each, obtaining coefficients of variation (CV) ranging from 2.9 to 6.1%. Reproducibility was checked with three different sera, which were assayed 12 times each by three different technicians. The CVs ranged from 6 to 7%.

Under our conditions, the mean total CK activity, derived from data on 32 normal, young adults, was 76 SD 35 U/L. The CK-MB isoenzyme was detected in only two of these normal sera, at 0.1% and 1.3% of the total activity (respectively, 0.20 and 0.80 U/L).

The highest measurable amount and the effects of dilution were studied in sera with a total CK activity >1200 U/L, before and after dilution with isotonic saline (Table 1). For undiluted samples, the CV exceeded 10%, whereas after dilution the results were fully reproducible.

Fluorescence of CK-MB is linearly related to activity in the range of 1 to 100 U/L (n = 7, r = .985). Over this limit, either the serum must be diluted with saline or only 2 μL applied. The serum must also be diluted when total CK activity exceeds 600 U/L, to avoid poor resolution of the isoenzymes.

Despite the short incubation interval, some NADPH diffuses from the cellulose acetate to the paper strips that contain a significant fluorescence. Fortunately, the intensity of fluorescence diffusing from every spot is proportional to the fluorescence of this spot. To demonstrate this relationship, we measured the fluorescence of cellulose acetate and paper strips obtained for 31 sera with total activities ranging from 250 to 2950 U/L.

At the level of every isoenzyme, the correlation calculated between the total fluorescence in the three strips and the fluorescence measured in the cellulose acetate strip alone was r = .984 (n = 31).

In the case of 40 patients suffering from myocardial infarction, the CK-MB peak appeared 16 to 20 h after the onset of clinical symptoms, slightly before the peak of total CK activity. The peak activities of CK-MB ranged from 8.0% to 27.1% of the total. The method was found to be particularly suitable for diagnosis in cases of clinically and (or) electrocardiographically suspected myocardial infarction having a total CK activity within the normal range. For instance, a patient with a total CK of 86 U/L had 7.2% CK-MB.

We did not observe any increase of CK-MB in 25 patients suffering from cardiovascular diseases without myocardial necrosis. For 20 patients with muscular injuries or limb crush the total CK was variably increased, whereas we found an increase of CK-MB in only three cases, with no value exceeding 2.1%, even in a case where the total activity was 7540 U/L.

We conclude that this fluorometric technique is reproducible, fast (six samples may be evaluated by one technician within 40 min), sensitive within a large range of activities (from 1 to more than 300 U/L), easy to perform, and inexpensive in a laboratory possessing a fluorometer equipped with a thin-layer plate recording attachment.

We are grateful to the members of the Cardiovascular Disease Unit (Pr. A. Bajolet), who furnished the pathological sera.

### Table 1. Influence of High Activities of Total CK and Dilution of Serum on CK-MB as Measured by the Electrophoretic Fluorometric Method

<table>
<thead>
<tr>
<th>Sample applied</th>
<th>Serum diluted 1/3 with saline</th>
<th>Serum diluted 1/5 with saline</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CK-MB, CV, U/L</td>
<td>%</td>
</tr>
<tr>
<td>Total CK acy, U/L</td>
<td>n</td>
<td>1269</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>1510</td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>1920</td>
</tr>
</tbody>
</table>

References


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Lactate Dehydrogenase isoenzyme 6 with Increased Creatine Kinase BB isoenzyme

To the Editor:

We recently observed a serum showing a definite LDH 6 band with an increase in CK-BB. The patient, a 77-year-old man, had suffered a concussion. The serum was sent to us from the hospital to which he had been admitted.

Serum total CK was 368 U/L (normal 0–178). Total LDH 2010 U/L (normal 100–225).

Cellulose acetate electrophoresis (Helena system) to resolve isoenzymes of CK showed 76% MM, 6% MB, and 18% BB.

The (Helena) LDH isoenzyme electrophoresis showed six well-differentiated bands: LDH₁ 10.4%, LDH₂ 14.9%, LDH₃ 18.0%, LDH₄ 19.1%, LDH₅ 30.2%, and LDH₆ 7.4%.

CLINICAL CHEMISTRY, Vol. 29, No. 7, 1983 1443
Previous reports (Clin Lab Letter/NEWS 4: 4, 1982) have confirmed that evidence of LDH 6 is associated with poor patient prognosis. Follow-up studies of this case were not possible, because the patient died.

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Influence of Minicolumn Procedure on Apparent Hemoglobin A1 in the Presence of Hemoglobin J-Baltimore

To the Editor:

Recently, we reported a normal column-chromatographic glycosylated hemoglobin (hemoglobin A1) in an individual heterozygous for the fast β-chain variant, hemoglobin J-Baltimore (αβ^1^2 Gly^→^Aep) (1, 2). Here we report on a non-diabetic patient whose apparent HbA1, when measured by a different minicolumn procedure, was increased by a scant amount of HbJ-Baltimore which had been received in a transfusion.

A 30-year-old black woman with a stroke was transferred to the Eugene Talmadge Memorial Hospital. Before transfer she had received multiple transfusions for anemia resulting from menometrorrhagia. She denied a history of diabetes mellitus.

Laboratory data obtained at admission included: Hb 136 g/L, mean cell volume 76 fl, reticulocyte count 1.6%, serum glucose concentration 4.5 mmol/L, and normal results for a urinalysis. Serum glucose did not exceed 5.88 mmol/L on two other occasions. Hemoglobin electrophoresis was ordered, in investigating the cause of the stroke.

Both hemoglobin electrophoresis on cellulose acetate (Helena Laboratories, Inc., Beaumont, TX 77704) and on starch gel at a reference laboratory revealed a small proportion (5.6%) of fast variant hemoglobin, evaluated by elution from cellulose acetate (3), having the mobility of HbJ-Baltimore. The apparent HbA1 was 10.2% by the Glycosylated Fast Fraction, Hemoglobin Quik Column (Helena Laboratories, Inc., Beaumont, TX 77704), but only 7.5% by the Fast Hb Test System (Isolab Inc., Akron, OH 44321). Two weeks later this fast hemoglobin was no longer detectable.

To study the cause of the different HbA1 results for the Helena and Isolab methods, we measured the effective length and volume of both minicolumns. These were 2.0 cm and 0.6 ml for the Helena column and 4.6 cm and 2.0 ml for the Isolab column. In the Helena method 1.5 ml of buffer is used to elute the HbA1, and in the Isolab method 4.0 ml. The ratio of fast hemoglobin elution buffer to resin volume is 2.5 for the Helena minicolumn, only 2.0 for the Isolab minicolumn.

We believe the microcytic anemia was caused by iron loss from chronic menometrorrhagia. The disappearance of the fast hemoglobin on electrophoresis two weeks later was compatible with its passive acquisition by transfusate. The increased apparent HbA1 with the Helena minicolumn was due to its contamination by the fast HbJ-Baltimore. The patient's lack of a history of diabetes and normal results for serum glucose and urinalysis are consistent with this conclusion. Only 5.6% fast HbJ-Baltimore increases the apparent HbA1 with the Helena system, but even 60% HbJ-Baltimore does not increase the Isolab HbA1 (1). This is because, as noted above, the Helena column is much shorter than the Isolab column and in the Helena method relatively more fast hemoglobin elution buffer is used than the Isolab method, thus causing contamination with HbJ. We believe that the Isolab HbA1 is more nearly accurate than the Helena HbA1, in the presence of HbJ-Baltimore, in agreement with Hammons et al. (4).

We thank Dr. Titus H. J. Huisman for hemoglobin variant identification.

References


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Faster Analysis for Total and Direct Bilirubin in the Cobas-Bio Analyzer

To the Editor:

Each day the clinical laboratory serving the neonatal population per-

Origin of Anodally Migrating Alkaline Phosphatase isoenzyme in Transient Hyperphosphatasaemia of Infancy

To the Editor:

We were misquoted by Weber et al. (1). We did not speculate that the alkaline phosphatase isoenzyme of unusual anodal electrophoretic migration we observed in the sera of patients with transient hyperphosphatasaemia of infancy might originate in the alimentary tract. What we did suggest was that it might occur in response to an infection of the alimentary tract (2). The two statements are quite distinct.

Wieme had previously presented evidence of a non-intestinal origin for the isoenzyme, and believed it to be derived from liver (3). Both suggestions have been supported by our own findings, and we have recently presented evidence that the isoenzyme is an anodally sialated alkaline phosphatase, possibly of hepatic origin (4). We would additionally take this opportunity of pointing out that, although total alkaline phosphatase activity in these sera is generally heat-labile (<20% residual activity after heating at 56°C for 10 min in all but three of 13 cases studied), the anodal isoenzyme may have heat stability similar to that of normal liver (αL) phosphatase.

References


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